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Investigation of self-care behaviours in a rat model of acute liver injury induced by paracetamol*

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ABSTRACT

Aims: Acute liver failure is a clinical condition characterized by sudden onset, high morbidity, and mortality in the absence of any known liver disease. The present study aimed to investigate the status of self-care, anhedonia, and anxiety-like behaviors in a paracetamol-induced acute liver injury (ALI) model.

Methods: Twelve adult male Wistar Albino rats were divided into two groups: a control group (n=6) and an acute liver damage group (n=6). The control group received 1 ml/kg physiological saline intraperitoneally (i.p.), while the ALI group received a single dose of 750 mg/kg paracetamol (i.p.). Behavioral tests, including the open field test (OFT), splash test, and sucrose preference test, were performed. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme levels were measured.

Results: AST and ALT levels were higher in the acute liver damage group than in the control group ($p<0.05$). In the ALI group, the frequency and duration of grooming decreased, and the grooming latency time increased ($p<0.001$). In the OFT, locomotor activity parameters decreased while anxiety-like behaviors increased ($p<0.05$). Sucrose solution consumption decreased to below 65% in the ALI group and was significantly reduced compared to the control group ($p<0.001$).

Conclusion: In the ALI model, self-care behavior patterns were negatively affected in duration and frequency. Similarly, locomotor activity and exploratory behavior were decreased, and anxiety-like behaviors increased in the open-field test. Anhedonia was also observed in the sucrose preference test. Further studies on ALI are needed to investigate the negative behavioral changes observed.

Keywords: Acute live injury, anhedonia, anxiety-like behaviors, self-care behavior, splash test, paracetamol

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INTRODUCTION

Acute liver injury (ALI) is a serious clinical picture with sudden onset and high morbidity and mortality.[1] ALI can cause different reasons, such as dose-dependent drug toxicity, Idiosyncratic (not dose-related) drug-induced liver injury, viral hepatitis, autoimmune hepatitis, metabolic diseases, Budd–Chiari syndrome, toxin-induced and pregnancy-related.[2] Increased serum concentrations of liver enzymes, decreased ammonia detoxification, impaired production of clotting factors, and hepatocyte destruction are significant problems exhibited in ALI. Increased cellular debris leads to activation of the immune system and increased inflammation. Kupffer cells, infiltrating bone marrow-derived macrophages and hepatic stellate cells (HSC), remove hepatocyte cellular debris. Activated HSCs release collagen to maintain tissue integrity in the damaged liver and contribute to scar tissue formation and fibrogenesis.[3]

Paracetamol (acetaminophen) is one of the most widely used non-prescription drugs. Paracetamol is used in an extensive age range, from pediatric to adult. Further, paracetamol is a cheap and accessible drug.[4] According to clinical investigations, approximately 28- 51% of ALI is due to acetaminophen overdose, which is the primary factor for etiology.[5,6] Paracetamol use is increasing in the United States and Western countries. Every year, there are hospitalizations due to acute paracetamol toxicity. People are experiencing acute paracetamol-induced liver toxicity, both intentionally (in particular suicidal) and due to misuse of doses. Patients may experience coagulopathy, encephalopathy, and even mortality.[6]

There is an essential link between liver and brain health. In particular, chronic liver diseases can lead to different psychiatric disorders.[7,8]

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According to preclinical studies, despair-like behavior and some anxiety-like behaviors were reported to be increased in the chronic liver disease model.[9,10]

The present study aimed to investigate behavioral changes during the early phase of ALI. From the point of view of translational medicine, it may be essential to determine which behaviors will be altered in the early period of paracetamol toxicity, which is the most common cause of ALI. For this purpose, different anxiety-like behaviors and especially self-care behaviors impaired in depression were examined.

METHODS

Animals and Experimental Design

12 adult male Wistar Albino rats were obtained from Balıkesir University Experimental Animal Laboratory. The groups were divided into a control group (n=6) and an ALI group (n=6). Approval was obtained from Balıkesir University Animal Experiments Ethics Committee (Date: 25.12.2023, Decision No: 2023/11-2). All experiments followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.[11]

An ALI model was performed with paracetamol. In the present study, a single dose of 750 mg/kg/day was administered intraperitoneally, as in Ishida et al.[12] and Mroueh et al.[13] Rats were sacrificed under a 50 mg/kg ketamine +10 mg/kg xylazine combination and exsanguinated by cardiac puncture. To confirm the model, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured in serum. AST (Otto Scientific, catalog no: OttoBC127) and ALT (Otto Scientific, catalog no: OttoBC128) were measured colorimetrically using the MINDRAY-BS400 device.

Open Field Test

The open field test (OFT) assesses general locomotor activity and anxiety-like behaviors.[14] The OFT is an animal anxiety test created in an open field and novel environment. The subject was taken to the experimental environment 2 hours in advance to adapt to the experimental environment. The environment was illuminated with a 100-110 lux lamp. The test apparatus was wiped with 70% alcohol after each experiment. In the experiment, the time spent in the central zone, the number of entries to the central zone, and the decrease in the behavior of getting up on both feet are indicators of increased anxiety. The number of transitions between squares dividing the open area is a parameter of horizontal locomotor activity. Fecal boli numbers are performed to examine autonomic dysfunction. All of these parameters were video recorded for 5 minutes.

Splash Test

Sucrose solution is sprayed on the dorsal part of the animals to trigger self-cleaning behavior. In this test, animals with increased depression-like behaviors are expected to have less self-cleaning behavior than control animals. The self-cleaning behavior seen in this test models self-care in humans. The duration and frequency of grooming decrease with increasing depression-like behaviors while grooming latency is prolonged.[15] The dorsal fur of the rat was sprayed with 1 ml of 10% sucrose solution at a time. This procedure was performed under red light (15 W) and video recorded for 5 minutes. The onset, duration, and frequency of scratching behavior were analyzed.

Sucrose Preference Test

To investigate the anhedonia behavior observed in depression in the subjects, a sugar preference test was performed.[16] A water solution containing 2% sucrose and tap water was placed in separate water bottles in each cage.[16] To prevent the formation of neophobia (fear of new objects), the bottles containing sucrose water and plain water were switched every 12 hours. The data obtained were calculated as the percentage of sucrose consumption. The positions of the bottles were changed to prevent the possible effect of side preference or neophobia. The total liquid volume consumed was recorded and used to assess the sucrose preference index. Sucrose preference was calculated using the formula [sucrose solution consumed volume/total consumed volume ×100).[14]

Harderian Gland Secretion Score

The Harderian gland is located in the orbit of the eye in different animals, including rats. During stress and disease, harderian gland secretion increases in rats, and discoloration occurs around the eyes and nose.[17] This situation is called Chromodacryorrhoea. Harderian gland function will be evaluated on a scale of 0-5 according to the Chromodacryorrhoea scale after ALI.[17] According to the Chromodacryorrhoea scale, score 0: No drops present; score 1: a single red-black drop of 1 mm or smaller; score 2: a drop larger than 1 mm or several small red-black drops; score 3: assigned when several large drops or numerous small red-black drops form, score 4: assigned when approximately 25-50% of the eye or nose is covered by red-black drops, score 5: Assigned when red-black drops cover more than 50% of the eye area or nose.[17]

Statistical Analysis

All data were evaluated for normal distribution using the Shapiro-Wilk test. Then, data analyses were performed using the Student's *t*-test. Mean±SEM represented the results of studies. Differences were admitted to be statistically significant at $p<0.05$. A priori power analysis was conducted using G*power 3.1 to determine the appropriate sample size for comparing two independent groups (Control vs. ALI) using a two-tailed t-test for independent samples, with the primary outcomes including grooming behavior, locomotor activity, and sucrose preference rate. Effect size (Cohen's d): 1.6 (a large effect, based on behavioral outcomes like grooming reduction and sucrose preference), α (alpha) error probability: 0.05, power (1-β): 0.80 (80%), and allocation ratio (n2/n1): 1 were performed. The total sample size required to detect a large effect size (d=1.6) with 80% power and 5% significance level is n=12 (6 rats per group). This analysis confirms that the sample size used in this study (n=6 per group) is adequate to detect large effect sizes, particularly in behavioral changes and biochemical alterations due to ALI induced by paracetamol.

Furthermore, in the Resource Equation Method for Animal Studies, two group designs with six subjects per group are sufficient.[18]

RESULTS

Biochemical Results

AST and ALT increased significantly in the ALI model induced by paracetamol compared to the control group (**Figure 1**). AST values showed a dramatic increase in the ALI group (120.5±13.96) compared to the control group (19.87±3.36) and increased approximately 6-fold ($p<0.001$). Similarly, ALT values significantly increased in the ALI group (22.63±2.45) compared to the control group (9.8±1.48) ($p<0.01$).

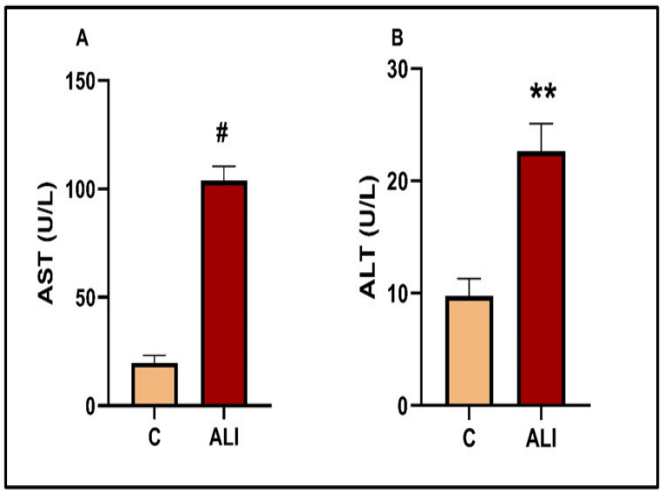

Figure 1. Molecular findings for the experimental groups. **(A)** Serum-AST, **(B)** serum-ALT. Results are presented as mean±SEMs (**p<0.01, #p<0.001)
C: Control, ALI: Acute liver injury, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Behavioral Results

Behavior parameters were analyzed for anxiety-like behaviors (ALB) and locomotor activity in the OFT, which are shown in **Figure 2**. According to the results of the OFT, total distance traveled ($0.05<p$), unsupported rearing number ($0.05<p$), and time spent in the center zone ($p<.0.05$) and center zone entrance number ($p<0.05$) were significantly decreased in the ALI group compared to the control group. Fecal boli numbers were not significant ($p>0.05$).

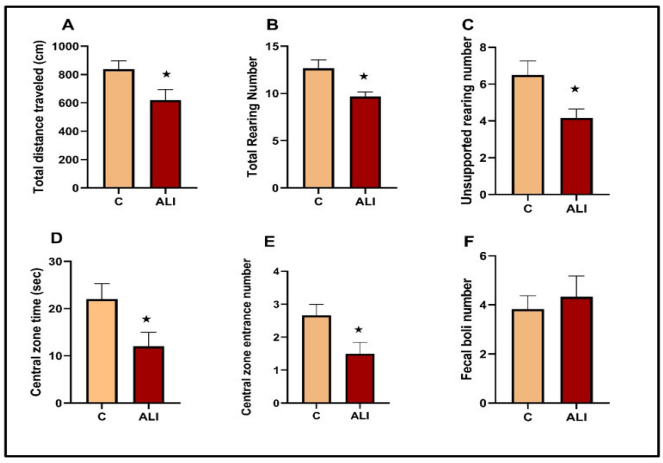

Figure 2. Behavioral findings in the open field test in the experimental groups. **(A)** Total distance traveled, **(B)** total rearing number, **(C)** unsupported rearing number **(D)** central zone time **(E)** central zone entrance number, **(F)** fecal boli number, values in the graphs are presented as the means±SEMs (*p<0.05)
C: Control, ALI: Acute liver injury

The sucrose preference percentages of the subjects decreased below 65% in the ALI group. The paracetamol-treated group significantly reduced sucrose preference ($p<0.001$). **Figure 3** in the sucrose preference test shows the results of anhedonia-like behaviors. Harderian gland score slightly increased in the ALI group but was insignificant ($p>0.05$) (**Figure 3**).

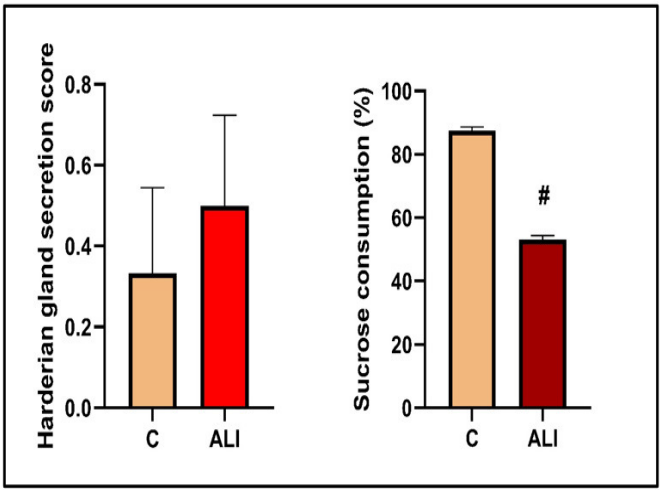

Figure 3. Behavioral finding in the sucrose preference test in the experimental groups. Sucorse solution consumption (%) values in the graph is presented as the means±SEMs (#p<0.001)
C: Control, ALI: Acute liver injury

Furthermore, self-care behavior patterns were observed in the splash test, as shown in **Figure 4**. The grooming time in the splash test ($0.001<p$) and grooming frequency ($p<0.001$) were significantly reduced in the ALI group vs the control group. The ALI group significantly prolonged grooming behavior latency ($p<0.001$).

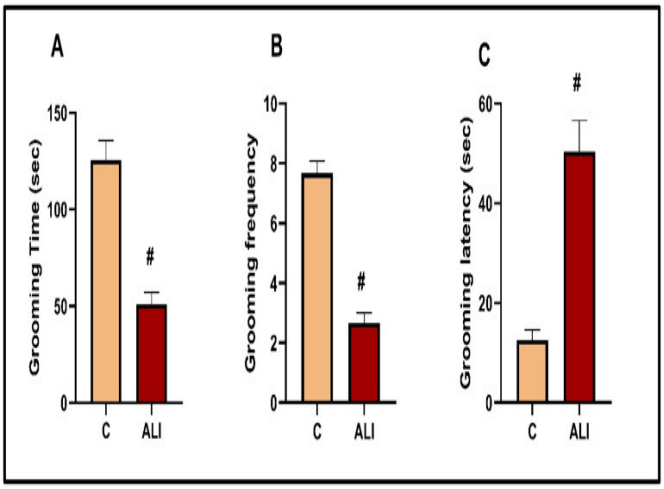

Figure 4. Behavioral findings in the splash test in the experimental groups. **(A)** Grooming time **(B)** grooming frequency **(C)** grooming latency, values in the graphs are presented as the means±SEMs (#p<0.001)
C: Control, ALI: Acute liver injury

DISCUSSION

The presented study established an ALI model with paracetamol, and early behavioral changes were investigated.

In the OFT, anxiety-like behaviors increased in subjects in the ALI group. Subjects spent time near the walls of the OFT and avoided from the central area. This behavior pattern, called

thigmotaxis, is an essential indicator of increased anxiety-like behaviors.[20] In the study presented, not only did the time spent by the subjects in the central area decrease, but the number of times they entered the central area also decreased. Yang et al.[21] reported cognitive impairment, increased anxiety-like behavior, and reduced locomotor activity in liver toxicity induced by thioacetamide in the young male Spraque Dawley rats. In the same study, central zone time, central zone entries, and total distance traveled decreased similarly to our findings.

A decrease in locomotor activity and exploratory behavior was reported to accompany inflammation, oxidative stress, and increased serum ammonia levels in the OFT.[22] In particular, proinflammatory cytokines may contribute to a decrease in exploratory behavior and locomotor activity. Further, liver toxicity disrupts the microbiota-gut-liver axis.[22] This axis can be examined in detail to clarify the pathophysiology.

Unsupported rearing, an exploratory behavior affected by anxiety, was examined in the OFT.[23] In the present study, research behavior was significantly suppressed and reduced. The locomotor activities of the subjects decreased along the horizontal axis. According to another preclinical study, the thioacetamide-induced mouse model of ALI also showed that locomotor activity decreased.[24]

In open-field test studies, cognitive defects have also been examined using other behavioral tests.[22] Liver toxicity impaired spatial memory has been reported in the Morris water tank.[22]

The splash test is a significant depression test that examines self-care behaviors.[15] In subjects with the ALI model, grooming, a self-care behavior, was significantly affected. Despite the dirt triggered by sugary water, the subject's grooming time and frequency decreased. The subjects also started cleaning and grooming themselves later. These findings suggest that self-care deficits associated with depression are observed in early-stage ALI. Furthermore, Anhedonia associated with depression was observed in the sugar preference test in the ALI group. Tap water consumption increased in the subjects due to their inability to derive pleasure.

The Harderian gland, which is associated with stress, may be an essential indicator of both psychological condition and animal welfare.[17] Harderian gland secretion scores were found to be high in acute LPS toxicity with severe neuroinflammation.[25] No association was found in the STZ-induced type-1 diabetes model.[23] No significant changes were observed in the present study's early phase of ALI. Significant changes may be observed in experimental designs where the long-term effects of ALI are examined.

Liver dysfunction causes an excessive accumulation of ammonia, which contributes to the disruption of potassium homeostasis, mitochondrial dysfunction, oxidative stress, inflammation, hypoxemia, and dysregulation of neurotransmission.[26] Ammonia has been reported to cross the blood-brain barrier.[27] Preclinical studies have shown increased astrocyte and microglia reactivity, inducible nitric oxide synthase, proinflammatory cytokines, and prostaglandin E_2 levels. These biomolecule changes cause alterations in the expression of neurotransmitter receptors on cell membranes and may also be responsible for impaired cognitive and motor functions.[26]

Neurotrophic factors have various functions, including regulating neuronal survival, neurotransmitter release, and dendritic growth. The production of neurotrophic factors may be adversely affected in psychiatric disorders.[25] There may be a link between neurotrophic factors and hyperammonia in mood disorders.[28] Suh et al.[28] reported that patients with acute toxic failure had high anxiety-depression tendencies. This tendency may be caused by hyperammonia disrupting the production of neurotrophic factors.

The serious problem of ALI is brain herniation, resulting in cerebral edema and associated intracranial pressure increase. Several factors involved in the pathogenesis of ALI have been shown to contribute to astrocyte swelling and brain edema. Oxidative stress, mitochondrial permeability transition, and Nuclear factor-kappaB (NF-κB) activation contribute to this process, affecting the water channel protein aquaporin-4. These changes in the fine structure of cells can cause brain dysfunction and behavioral disorders.

Further preclinical and clinical studies will shed light on the connection between the liver and the brain.

Limitations

The study was conducted on male rats. Due to budget and time constraints, the study was conducted on male rats. Preclinical studies may also be essential to perform on female rats, as this reflects the translational medicine and public health aspects of the research. In the presented research, serum ALT and AST levels were examined to validate the model. Biochemical tests used in bleeding and clotting assessment can also be employed. Histological evaluations may also serve as an additional validation method.

CONCLUSION

Liver enzymes were elevated in the serum due to damage caused by paracetamol. In the early stage, impaired self-care behaviors, anhedonia, increased anxiety-like behaviors, and decreased locomotor activity were observed in the ALI model. No change was observed in the harderian gland secretion, which indicates stress and animal welfare. From a translational medicine perspective, self-care, anhedonia, locomotor activity, and mood changes can be assessed in clinical practice for early signs of liver damage.

ETHICAL DECLARATIONS

Ethics Committee Approval

Approval was obtained from Balıkesir University Animal Experiments Ethics Committee (Date: 25.12.2023, Decision No: 2023/11-2).

Informed Consent

The informed consent form was not obtained because of the study's experimental design.

Referee Evaluation Process
Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
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Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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